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(54) **Identification of microorganisms by infrared analysis of evolved carbon dioxide.**

(57) A method and apparatus for the identification of a microorganism in a sample is provided. The microorganism is exposed to conditions conducive to growth in the presence of a plurality of growth substrates which are individually inoculated with the microorganism. The presence or absence of carbon dioxide as a by-product of the metabolism of these substrates is detected by infrared analysis and provides a profile of the unknown microorganism. Identification is accomplished by comparing this profile with profiles of known microorganisms treated in the same way.

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IDENTIFICATION OF MICROORGANISMS BY INFRARED ANALYSIS OF EVOLVED CARBON DIOXIDE

Field of the Invention

The present invention relates generally to the identification of microorganisms. More particularly, the present invention relates to the identification of an unknown microorganism by comparison of the carbon dioxide evolution profile of the unknown microorganism generated from growth in a plurality of substrates with profiles of known microorganisms generated from growth in the same plurality of substrates.

Description of the Prior Art

Conventional methods of organism identification usually involve culturing techniques. In general, this procedure consists of inoculating a sample in a suitable growth medium and growing any microorganisms present in the sample to a concentration such as to allow plating of the microorganism onto a solid agar medium. The microorganism is subsequently recovered as a relatively pure concentrated sample. The microorganism is then identified as to colonial morphology and Gram reaction by observation under a microscope. Culture techniques are time consuming and are often not completely definitive. There is a need for a more rapid and definitive means for microorganism identification.

The generation and use of profiles of standard microorganisms generated from a plurality of substrates for the identification of unknown microorganisms is known. A commercial system for

identification of bacteria from clinical samples is available under the trademark SCEPTOR from BBL Microbiology Systems division of Becton, Dickinson and Company. In the use of this system, an unknown speciman is incubated in a plurality of individual growth substrates in separate wells of a panel. Metabolic activities such as oxidation of carbohydrates or deamination or decarboxylation of amino acids result in color changes in those wells in which the microorganism utilizes the substrate in that well. Identification is accomplished by comparing a profile of the metabolic activities of the unknown microorganism with profiles of various known microorganisms.

Another system for identification of microorganisms which utilizes the metabolism of Carbon-14 labeled substrates to produce radiolabeled carbon dioxide is disclosed in United States Patents 3,946,496 and 4,057,470 to Schrot. In the Schrot method, the evolved $^{14}CO_2$ is collected and assayed by nuclear counting means in order to construct radiorespirometric profiles. The method disclosed in the Schrot patents requires expensive radiolabeled substrates and nuclear counting equipment. In addition, it is necessary to count the $^{14}CO_2$ evolved from each substrate for a period of time sufficient to develop detection and rate data.

Infrared detection of CO_2 in head space gas as a means of determining microbial contamination in commercial food products has been described by C. H. Threlkeld (J. of Food Science, 47, 1225 (1982). This

method, however, deals only with detection and is not concerned with identification.

It is thus the principal object of the present invention to provide a rapid means for the identification of microorganisms. Another object of the invention is to provide a means for rapid organism identification which does not depend upon radiolabeled substrates. It is an additional object of the invention to provide identification system for microorganisms which does not depend upon chemical indicators or other added indicating reagents to classify reaction results. It is a further object of the invention to provide a process amenable to automated detection means so as to eliminate inconsistencies present when reaction results are interpreted by the human eye.

Summary of the Invention

The present invention provides an apparatus and method for identification of microorganisms in a sample, such as blood, urine, spinal fluid, water and the like. The invention comprises inoculating a plurality of substrates with a sample of the unknown microorganism. The substrates include various different carbon sources, such as carbohydrates and amino acids, as well as other components required for growth of the microorganism. Each of the substrates may be metabolized by certain specific microorganisms. The inoculated substrates are incubated for a predetermined length of time sufficient to permit

metabolic breakdown of some of the carbon source to produce carbon dioxide. The occurrence of metabolism of each carbon source by the microorganism to carbon dioxide is determined by infrared analysis of the gas produced by metabolism of any microorganism present in the sample. The detection of carbon dioxide is taken as a positive indication of metabolism of the carbon source. Similarly, the failure of the unknown organism to produce carbon dioxide in amounts detectable by infrared analysis is taken as a negative indication of metabolism of that specific carbon source. A profile of the unknown organism is obtained by noting the positive and negative metabolic results obtained using a plurality of carbon sources. The profile thus obtained is compared to profiles obtained from known microorganisms. The unknown microorganism is identified by the correspondence of its profile with that of one of the known microorganisms.

It should be understood that the profiles generated for known microorganisms by the method of this invention may differ from profiles generated by other methods for the same organisms. Other methods utilize acid-base indicators present in the sample to indicate positive reactions by color change. In these methods, fermentation reactions are detected by acid production and subsequent drop in pH to indicate positivity, while decarboxylation reactions are detected by a rise in pH. Carbon dioxide may or may not be produced as a consequence of these or similar metabolic reactions.

The process of the invention may be conducted in an apparatus which comprises a container for a plurality of different and separate carbon sources, means for inoculating each of the containers with a sample of the unknown microorganism, means for exposing the inoculated containers to conditions conducive to the occurance of normal metabolic processes, and infrared means for analyzing carbon dioxide gas produced as a result of metabolism of the carbon sources to determine whether or not metabolism occurred in each specific growth medium.

A Brief Description of the Drawings

Fig. 1 shows an apparatus embodying the principles of the invention.

Fig. 2 shows the infrared absorption spectrum of carbon dioxide and the absorption band between 2300 and 2400 cm^{-1}

Figs. 3, 4 and 5 show the infrared absorption spectrum of carbon dioxide in the area of interest for polymethylpentene, borosilicate glass and soda lime glass containers respectively.

Figs. 6 and 7 represent the infrared scan of polymethylpentene plastic and borosilicate glass containers which contain various levels of carbon dioxide.

Figs. 8 and 9 show carbon dioxide production from the metabolism of microorganisms in various growth media.

Figs. 10 and 11 show positive and negative carbon dioxide production by microorganism metabolism of various carbon sources.

Detailed Description of the Invention

An apparatus suitable for the practice of the invention is designated in Figure 1 by the numeral 11. In apparatus 11 of the present invention, the presence or absence of CO_2 produced by metabolism of a microorganism in a growth medium is detected by passing an infrared beam through the sidewall of a container and detecting the infrared absorption of the gas produced by metabolism within the container. The container may be a vial or any other single or multi-channel container.

A sample to be analyzed, such as a patient speciman, for example, blood, urine, or the like, or an organism grown in culture from a variety of sources (water, soil, food stuffs, patient specimans, etc.), is placed into a sterile container, 13, together with a growth medium suitable to produce CO_2 upon metabolism of a microorganism. Thereafter the sample is incubated in the container. At suitable intervals the container is transferred to an infrared measuring means which includes an infrared source 15 and an infrared detector 17. The infrared absorption within the head space of the container is detected and may be displayed on a meter 19 which may include provision for simultaneous display of a light signal 21 and a computer printout 23.

The container 13 is located on a track 25, which may be linear, circular, or of serpentine geometry to provide easy positioning of the container 13 between the infrared source and the infrared detector. A second culture container 13 A is shown in phantom outline in position waiting to be tested by infrared

source 15 and infrared detector 17. A motor 27 is provided to activate the track 25. A sequence controller 29 is used to properly sequence activation of the motor 27, the infrared source 15 and the meter display 19. It may sometimes be desirable to provide means for rotating a cylindrical container during activation of the infrared source to provide compensation for uneven sidewall thickness and additionally to provide means for aligning the containers in the center of the infrared beam.

The culture containers 13 and 13 A may be glass or plastic containers, such as vials, fitted with rubber septa and sealed by means of aluminum closures, or may be separate but contiguous wells of a molded plastic cartridge or tray of linear geometry. Other materials and geometry satisfactory for the performance of the required gas analysis may be employed if desired. The containers will have a total capacity of between about 1 ml and about 200 ml, preferably between about 30 ml and about 150 ml, of which preferably 2-100 ml will be occupied by the culture medium and test sample. The volume of blood or urine or other sample may be, for example, 0.1-10 ml.

The container must have a "window" providing at least about one percent transmittance at the band width suitable for detecting the gaseous product from metabolism of the growth medium. Carbon dioxide has a strong infrared absorption band at 2349 CM^{-1}, shown in Figure 2, free from water vapor interference. The required transparent window of the container when using this absorption band thus includes wave numbers from about 2300 CM^{-1} to about 2400

CM^{-1}. Carbon dioxide also has a low frequency absorption band at 670 CM^{-1} which is of use in the practice of this invention.

It has been discovered that certain types of glass containers and a particular type of plastic container are useful in the practice of the present invention. In particular, borosilicate glass containers and soda lime glass containers have been found to be useful. It has also been determined that polymethylpentene plastic provides a transparent "window" at the absorption wavelength of carbon dioxide. Other glass or plastic materials may also be used.

It should be understood that those skilled in the art of infrared spectroscopy do not consider either glass or plastic to be of use as a sample-containing cell for infrared spectroscopy in the usual infrared absorption range of 400 CM^{-1} to 4000 CM^{-1}. Glass, although transparent to visible light, becomes opaque to infrared wavelengths just slightly longer than those applicable to carbon dioxide analysis. Plastics, being organic materials, have infrared absorption bands throughout the usual range. It is surprising that polymethylpentene exhibits a transparent "windows" at 2349 CM^{-1} and near 600 CM^{-1} with enough transmission to permit the analysis of carbon dioxide at either of the above absorption frequencies.

While not wishing to be bound by any theory, it appears that carbon dioxide absorbs infrared energy in a wave length region corresponding to the covalent triple bond of organic molecules. Triple bonds are

not normally found in polymeric materials. Polymethylpentene is a preferred polymeric material containing covalent double bonds. For use as a container in the method of the invention, the polymeric material must also be capable of being sterilized, whether by conventional autoclave or gas or radiation sterilization techniques. Polymethylpentene is suitable in this respect in that it can withstand temperatures suitable to achieve sterility.

The growth medium which is to be inoculated with the microorganism may be composed of the single carbon source and a basal medium containing salts, buffers, growth factor(s) and the like. Alternatively, in instance where the buffering capacity and ionic strength of the medium is not of consequence, and where growth factor enhancement is not required, one or more of these components may be left out of the medium. Exemplary of suitable basal media which may be used for the practice of this invention are Carbon Assimilation Medium, hereinafter referred to as CAM, SCEPTOR Gram-negative MIC-ID dehydrated culture medium, hereinafter referred to as DCM, E. Coli basal medium 1, and Ledbetter medium. Such standard media are described in the Manual of Clinical Microbiology, Third Edition, American Society for Microbiology, 1980, edited by E. H. Lennette, E. H. Spaulding and J. P. Truant; in Experiments in Molecular Genetics, 1968 and 1972, edited by R. C. Cloroes and W. Hayes, Blacknell Scientific Publications; and in P. E. Goldenbaum and G. A. Hall, J. Bacterial. 140, p. 459.

The basal media may be prepared and the required carbon sources added prior to being dispensed into

the container, or the carbon sources may be present in the containers or cartridge wells in liquid, gel, frozen, elution disk or other dehydrated form and the basal medium added thereto.

Among the carbon sources which may be used are carbohydrates, such as glucose, lactose, arabinose, raffinose, sucrose, rhamnose, trehalose and the like; carbohydrate derivatives such as salicin; acid sugars; urea; polyols such as glycerol, mannitol, inositol, sorbitol, dulcitol, adonitol and the like; Krebs cycle intermediate acids such as citric acid; amino acids such as arginine, glycine, alanine, lysine, ornithine, tyrosine, threonine, histidine, leucine and the like; low molecular weight peptides; purine and pyrimidine bases; and low molecular weight compounds capable of being decarboxylated to produce carbon dioxide, such as fatty acids. The carbon source should be present in sufficient quantity to provide a readily detectable amount of carbon dioxide upon metabolism. Preferably, the growth medium contains from about two percent to about ten percent, on a weight basis, of the carbon source.

The growth medium containing the carbon source in the container is inoculated with a small amount of a sample containing the microorganism. Inoculation may be performed either manually or by automated means to prepare either a multiplicity of containers or multiple compartments within a common vessel.

After the inoculation step, the microorganisms are incubated at a temperature of between about 20°C and about 45°C, for a period of from about 1 to about 24 hours depending on the microorganism and the carbon source, but preferably for from about 2 to

about 8 hours. Some organisms achieve optimum growth at temperatures of 20°C or lower while others may exhibit optimum growth at 45°C or higher. Any temperature and duration of incubation best suited in a given circumstance may be employed. Although satisfactory growth can be achieved without agitation, metabolism preferably is carried out with active shaking, stirring, or the like, effective to insure proper evolution of CO_2 from the medium. In one preferred embodiment, agitation is provided by stirring or rotary shaking to introduce a vortex in the liquid medium.

Determination of the presence of CO_2 is effected by infrared analysis of the gas evolved from the culture medium. The absorption of infrared radiation at about 2360 cm^{-1} is used for this analysis. This absorption by CO_2 is shown in the infrared absorption spectrum of CO_2 in Figure 2.

In the practice of this invention, infrared absorption is determined immediately after inoculation and periodically thereafter up to the conclusion of the incubation period. Total absorption at about 2360 cm^{-1} is measured. Corrections for absorption by the containers are then made by measuring absorption of glass containers at 2400 cm^{-1} and of polymethylpentene containers at 2250 cm^{-1}. Subtraction of the reference absorptions of the containers from the total absorptions measured provides the corrected absorption due to the evolved CO_2.

A profile of the utilization or non-utilization (as determined by the presence or absence of carbon dioxide in the head space gas) of the carbon sources

is used to generate a profile of the unknown microorganism in the sample. A series of profiles of known microorganisms are similarly prepared. Comparison of the profile of the unknown with the known profiles permits identification of the unknown. It is evident that this comparison is facilitated by first separating the profiles of the unknown and the the known organisms into groups according to standard known classifications, such as the Gram stain, i.e., gram positive or gram negative.

The following examples further illustrate various features of the invention but are intended to in no way limit the scope of the invention.

Example 1

The relative amounts of infrared transmission through threee different materials is shown in Figures 3 to 5. In Figure 3, a 125 ml polymethylpentene bottle with the sidewall thickness of approximately .03 inches and an outside diameter of 1.88 inches is subjected to an infrared scan. The area of CO_2 absorption is outlined in Figure 3. As indicated,

the area of CO_2 absorption varies from a transmittance of from about 2 percent to about 7 percent. This is sufficient to permit a scan for CO_2 to be made. In Figure 4, a tubing vial of borosilicate glass with a sidewall thickness of 0.053 inches and an outside diameter of 1.33 inches is scanned at the wave numbers of interest. It is seen that the transmittance of borosilicate glass in the infrared wave number region slightly beyond the area of CO_2 absorption is zero. The percent transmittance in the area of CO_2 absorption varies from about 7 percent to about 14 percent. In Figure 5, a fifty milliliter bottle made of soda lime glass having an outside diameter of approximately 1.7 inches is scanned by infrared. Again, the percent transmittance at infrared wave numbers slightly greater than that for CO_2 absorption is zero. In the area of CO_2 absorption the percent transmittance ranges from about 3 to about 9. All infrared scans in this example and other examples were made with a Nicolet 5-MX FT-IR spectrophotometer. The scans for Figures 3 through 5 were made with the vials opened to room air and are the result of the addition and averaging of sixty scans. The scans are referenced against the scan of room air, which was also scanned sixty times. In each case the area of interest in terms of carbon dioxide absorption is in the region between approximately 2400 to 2200 CM^{-1}.

Example 2

In Figures 6 and 7, various levels of carbon dioxide gas were provided in the head space of the polymethylpentene bottle and a borosilicate glass

tubing vial respectively. Reference scans of each container were first taken with the bottles opened to room air by taking sixty readings in the Nicolet spectrophotometer. The vials were then flushed with gases containing approximately 2.5 percent, 5.0 percent and 10.0 percent carbon dioxide and were then quickly sealed. The bottle and the vial were then again read following each flushing for sixty scans in the Nicolet spectrophotometer. The results are shown in Figures 6 and 7 with the vertical axis in this case being the infrared absorbance times 100. A clear correlation can be seen between the increase in infrared absorption in the area between 2400CM^{-1} and 2300CM^{-1} with the increase in carbon dioxide concentration within the vials.

Example 3

The increase in infrared absorption as correlated with organism growth is shown in Figure 8.

30 Mls of tryptic soy broth (TSB) contained in two polymethylepentene bottles was sterilized by autoclaving. One bottle was inoculated at 0 hours with approximately one-half ml of a culture which had been grown overnight. The other bottle was not inoculated and was used as a reference. The bottles were scanned sixty times each at the intervals indicated in Figure 8. The data presented in Figure 8 represent the scan of the inoculated bottle minus the scan of the uninoculated bottle. The bottles were incubated at 37°C between readings. A clear correlation can be seen between organism growth and infrared absorption in the area of carbon dioxide absorption.

Figure 9 shows a scan of a soda lime glass vial containing sterile medium and a scan of a soda lime vial containing sterile medium inoculated with Clostridium perfringens. Both infrared scans were referenced against a scan of a soda lime glass vial open to room air. All scans were performed sixty times in the Nicolet spectrophotomer. The vial containing C. perfringens exhibits a much larger carbon dioxide signal than the vial containing the culture medium alone.

Example 4

Vials of soda lime glass containing 30 ml of either CAM or DCM and selected carbon sources were inoculated with 5 ml of a suspension of approximately 10^9 cells per ml of E.coli in normal saline. The infrared absorption was measured at time zero. Incubation was maintained at 37°C and the infrared absorption was measured after 5 hours. The results of this experiment are shown in Figures 10 and 11. Carbon dioxide was evolved and detected in the evolved gas as a result of metabolism by the microorganism of glucose, lactose, mannitol and lysine. The organism did not metabolize sucrose or inositol.

While the method and apparatus of the present invention have been described with respect to the detection of bacteria, the method and apparatus may

be used to detect biological activity broadly, including cell organisms, tissue culture cells, yeasts, enzymatic reactions and the like. Also, this technology may be applied to the assessment of the susceptibility of a microorganism to an antimicrobial agent, wherein the production of carbon dioxide by the organism may either increase or decrease in response to the effect of the agent on the organism's growth.

M&C FOLIO: 799P48693 WANGDOC: 0204R

CLAIMS

1. Apparatus for identification of a microorganism in a sample, the apparatus comprising:

a plurality of containers (13) each adapted for receiving an inoculating amount of the sample and a respective growth medium, each growth medium comprising a carbon source which might be metabolizable to produce carbon dioxide, the carbon sources being different in each of the containers and the containers being of a material permitting infrared detection of carbon dioxide;

means for incubating the growth medium and sample in the containers under conditions conducive to the occurence of normal metabolic processes so as to encourage gas evolution during the incubation;

means (15) for passing an infrared beam through the evolved gas in each of the containers and infrared detection means (17) for infrared detection of the presence of carbon dioxide in the respective evolved gas, in order to permit preparation of a carbon dioxide profile for the microorganism in the sample; and

a plurality of profiles of known microorganisms for comparison with the profile of the microorganism in the sample, the known profiles indicating utilization or non-utilization of the carbon sources by the known microorganisms, as determined by infrared detection of the formation or lack of formation of carbon dioxide.

2. Apparatus in accordance with Claim 1 wherein the containers have an infrared transmittance of at least one percent at a wave number region of from 2300 CM^{-1} to 2400 CM^{-1}.

3. Apparatus in accordance with Claim 1 or 2 wherein the containers are of borosilicate glass, soda lime glass or polymethylpentene.

4. Apparatus in accordance with Claim 3 wherein the containers are of borosilicate glass.

5. Apparatus in accordance with Claim 3 wherein the containers are of soda lime glass.

6. Apparatus in accordance with Claim 3 wherein the containers are of polymethylpentene.

7. Apparatus in accordance with any preceding Claim

wherein detection of carbon dioxide in the containers is effected sequentially and the apparatus further comprises means to align the containers in the centre of the infrared beam.

8. Apparatus in accordance with any preceding Claim, further comprising means to rotate the containers during the infrared detection of any evolved carbon dioxide.

9. A method for identification of a microorganism in a sample, the method comprising:

(i) providing a plurality of containers wherein individual containers are each provided with a different growth medium for microorganisms, each growth medium including a respective carbon source which might be metabolizable to produce carbon dioxide;

(ii) preparing a standard carbon dioxide profile for a particular known microorganism by the follwing steps:

(a) inoculating the plurality of containers with a sample of the known microorganism,

(b) incubating the container for a time sufficient to cause metabolic breakdown of the growth media thereby resulting in the production of carbon dioxide from at least some of the growth media,

(c) detecting the presence or absence of carbon dioxide in the gaseous atmosphere evolved from each growth medium by measuring the infrared absorption of the gaseous atmosphere by passing an infrared beam through the gaseous atmosphere and detecting the infrared absorption of the gaseous atmosphere,

(d) preparing a standard carbon dioxide profile for the known microorganism based on the utilization of growth media wherein the presence or absence of carbon dioxide is determined by infrared absorption;

(iii) repeating steps (a) through (d) for a plurality of known microorganisms to provide a series of standard carbon dioxide profiles for known microorganisms;

(iv) repeating steps (a) through (d) for a sample

containing an unknown microorganism to provide an unknown carbon dioxide profile; and

(v) comparing the unknown carbon dioxide profile with the series of standard carbon dioxide profiles with a view to identifying the unknown microorganism.

10. A method in accordance with Claim 9 wherein the infrared absorption is measured in glass containers at about 2360 CM^{-1} and at about 2400 CM^{-1}, and the absorption at about 2400 CM^{-1} is subtracted from the absorption at about 2360 CM^{-1} thereby to provide a corrected measurement of the absorption at about 2360 CM^{-1} due to carbon dioxide.

11. A method in accordance with Claim 9 wherein the infrared absorption is measured in polymethylpentene containers at about 2360 CM^{-1} and at about 2250 CM^{-1}, and the absorption at about 2250 CM^{-1} is subtracted from the absorption at about 2360 CM^{-1} to provide a corrected measurement of the absorption at about 2360 CM^{-1} due to carbon dioxide.

12. A method in accordance with Claim 9 wherein the infrared absorbance is measured at about 670 CM^{-1}.

13. A method in accordance with any of Claims 9 to 12 wherein the incubation is for 1 to 24 hours.

14. A method in accordance with Claim 13 wherein the incubation is for 2 to 8 hours.

15. A method in accordance with any of Claim 9 to 14 wherein the carbon source is selected from a carbohydrate, a carbohydrate derivative, a polyol, urea, citric acid, a fatty acid, an amino acid, a low molecular weight peptide, or a purine or pyrimidine base.

16. A method in accordance with Claim 15 wherein the carbon source is a carbohydrate.

17. A method in accordance with Claim 16 wherein the carbohydrate is selected from glucose, lactose, arabinose, raffinose, sucrose, rhamnose, or trehalose.

18. A method in accordance with Claim 15 wherein the carbon source is an amino acid.

19. A method in accordance with Claim 8 wherein said amino acid is selected from arginine, ornithine, lysine, glycine, alanine, tyrosine, threonine, histidine, or leucine.

20. A method in accordance with Claim 15 wherein the carbohydrate derivative is a salicin or the polyol is selected from glycerol, mannitol, inositol, sorbitol, dulcitol or adonitol.

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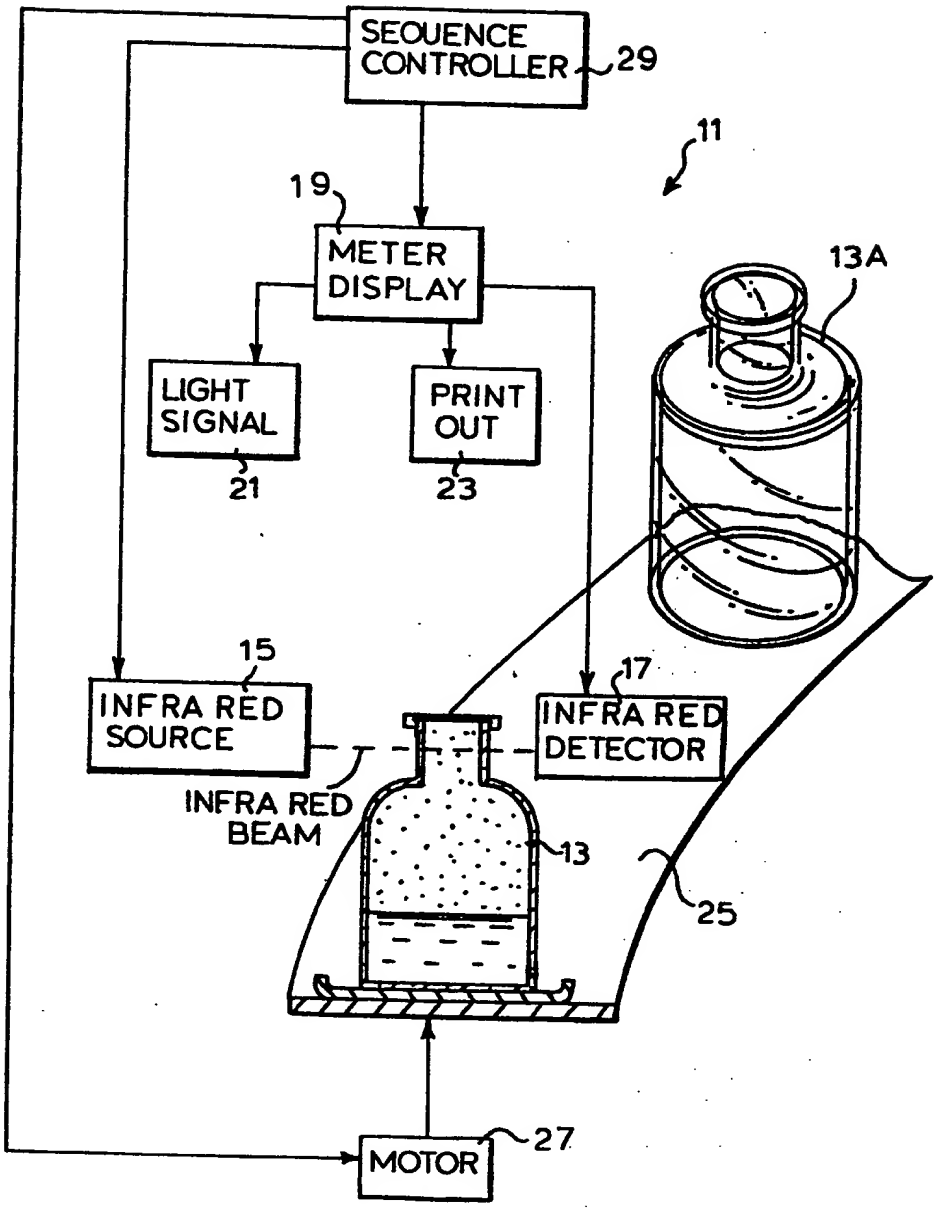


FIG.1

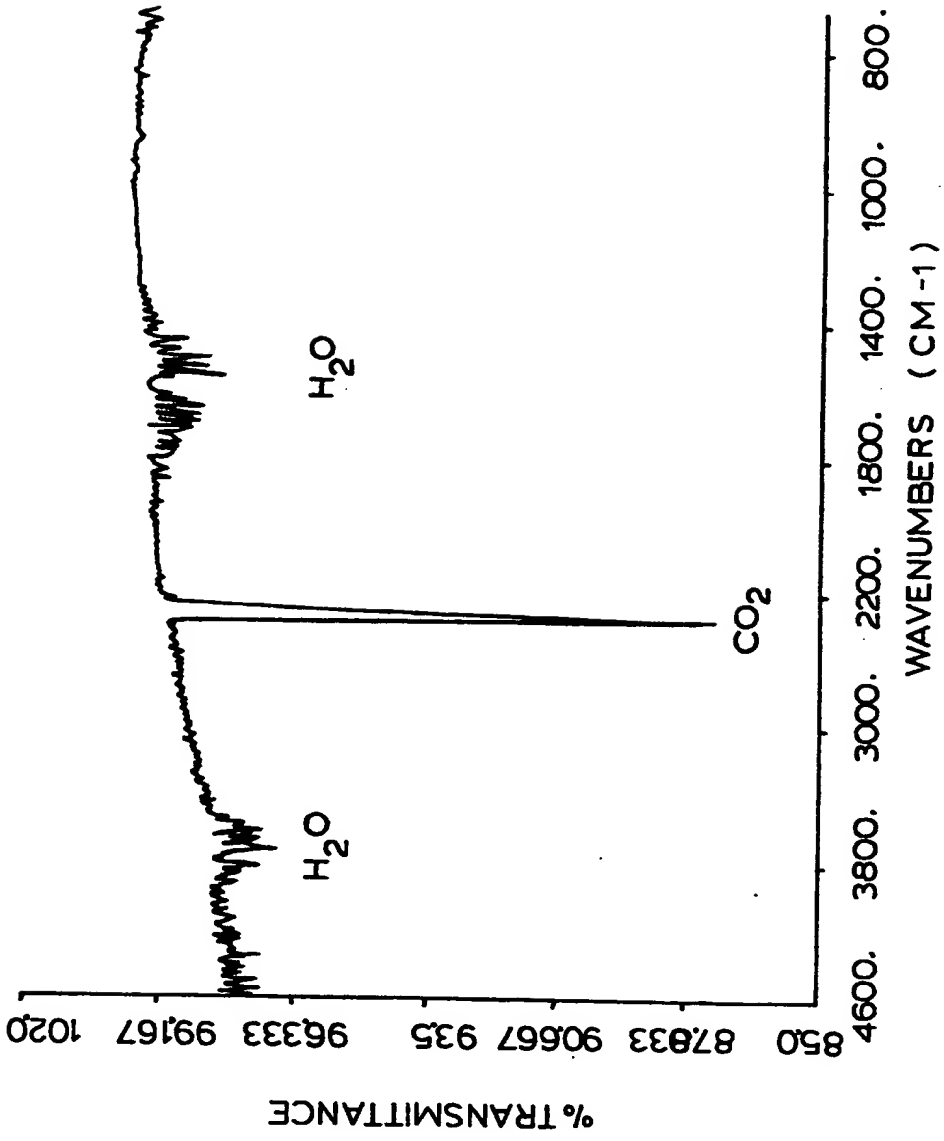

%TRANSMITTANCE
850 87833 90667 935 96333 99167 1020
4600. 3800. 3000. 2200. 1800. 1400. 1000. 800.
WAVENUMBERS (CM-1)
H_2O
CO_2
H_2O

FIG.2

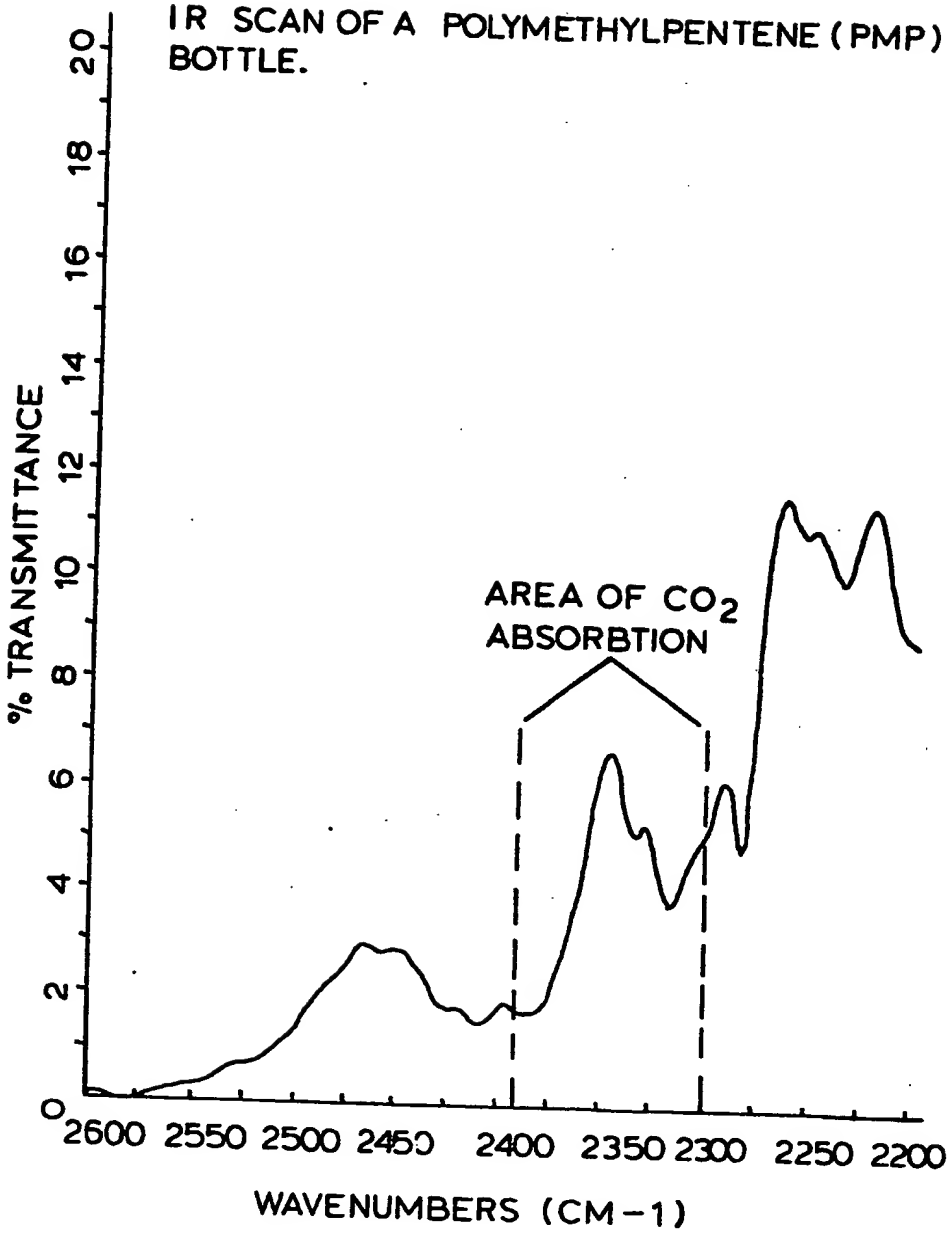

IR SCAN OF A POLYMETHYLPENTENE (PMP) BOTTLE.
% TRANSMITTANCE
0
2
4
6
8
10
12
14
16
18
20
AREA OF CO_2 ABSORBTION
2600 2550 2500 2450 2400 2350 2300 2250 2200
WAVENUMBERS (CM-1)

FIG.3

IR SCAN OF A BOROSILICATE TUBING VIAL

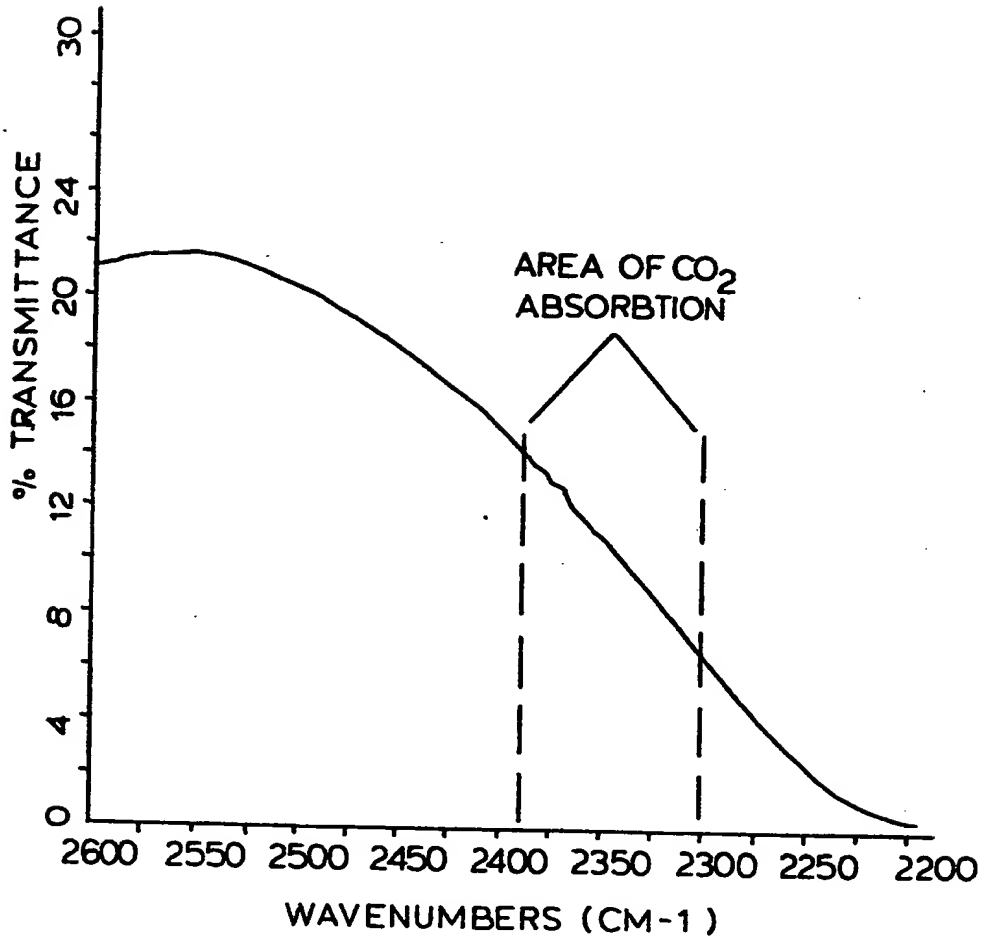


FIG.4

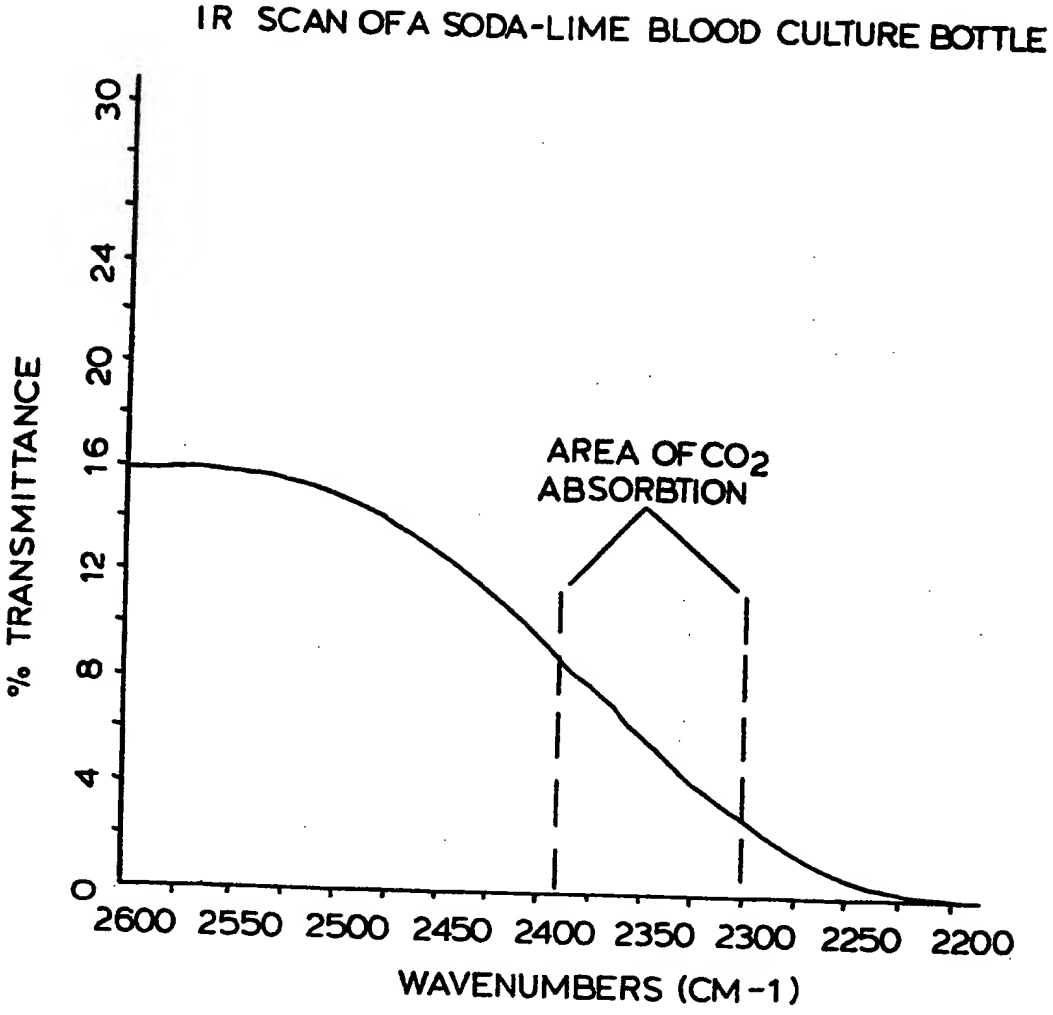

IR SCAN OF A SODA-LIME BLOOD CULTURE BOTTLE
% TRANSMITTANCE
0
4
8
12
16
20
24
30
AREA OF CO_2 ABSORBTION
2600 2550 2500 2450 2400 2350 2300 2250 2200
WAVENUMBERS (CM-1)

FIG.5

CARBON DIOXIDE QUANTIFICATION
IN POLYMETHYLPENTENE BOTTLE

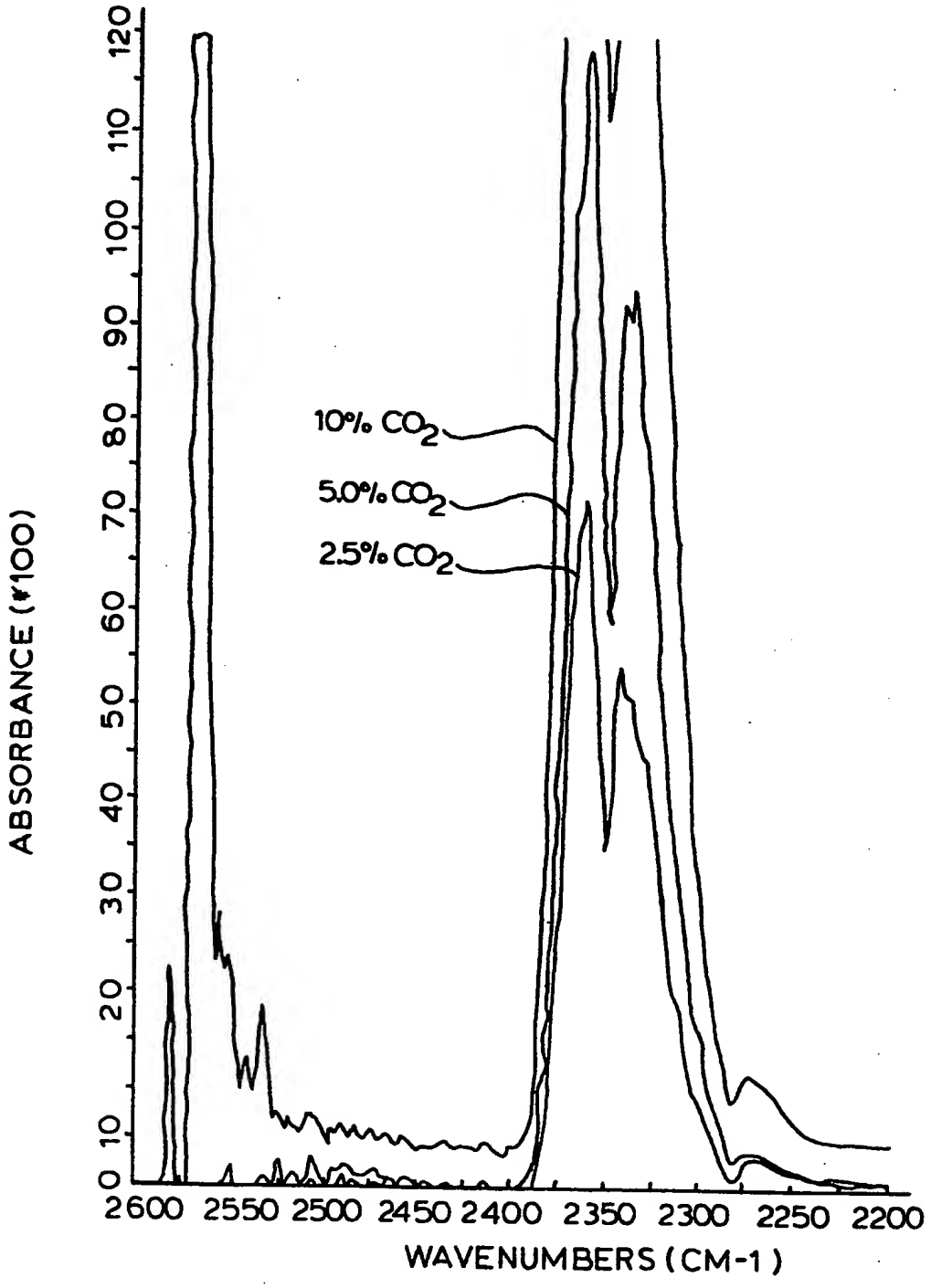


FIG.6

CARBON DIOXIDE QUANTIFICATION IN A BOROSILICATE GLASS TUBING VIAL

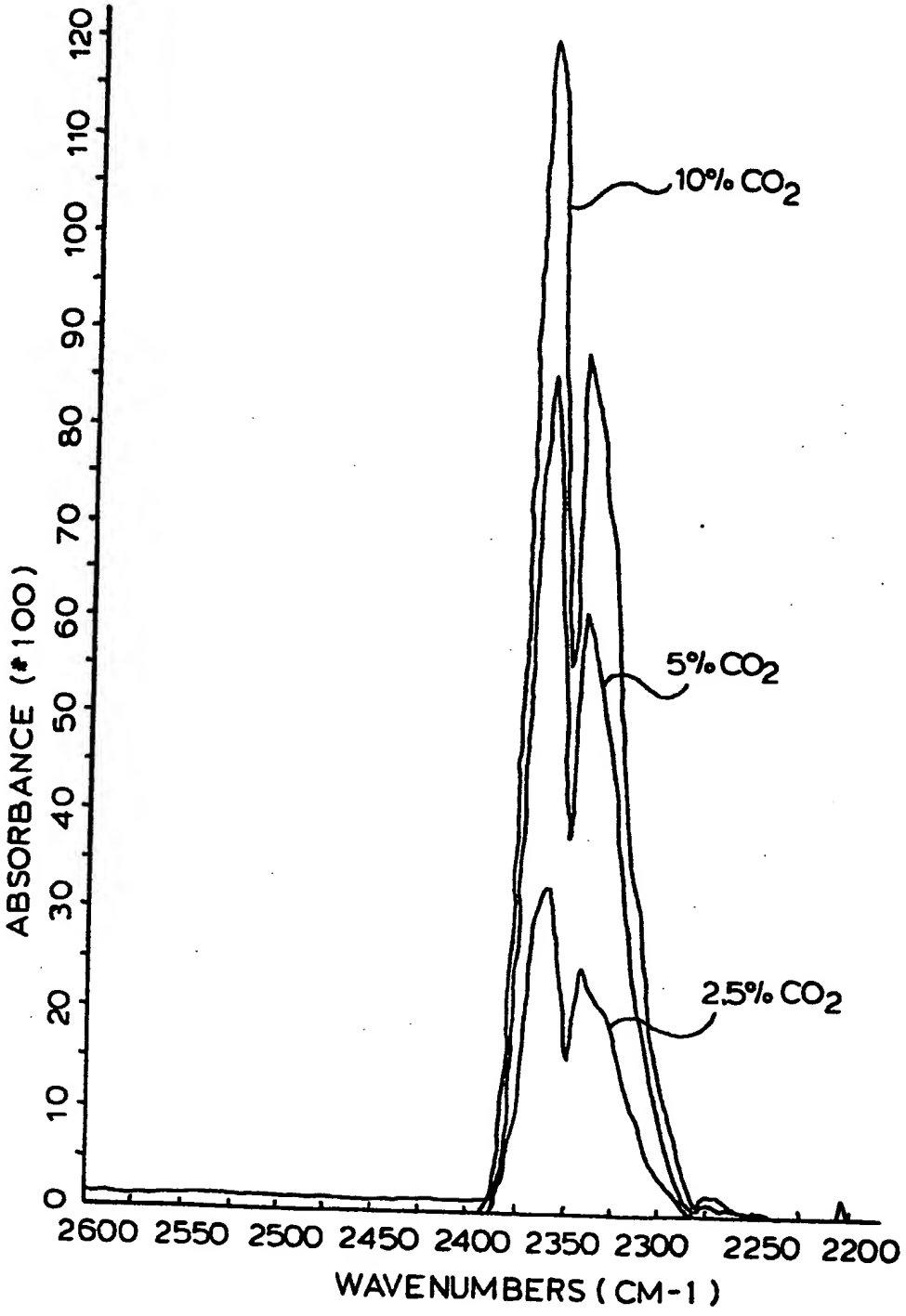


FIG.7

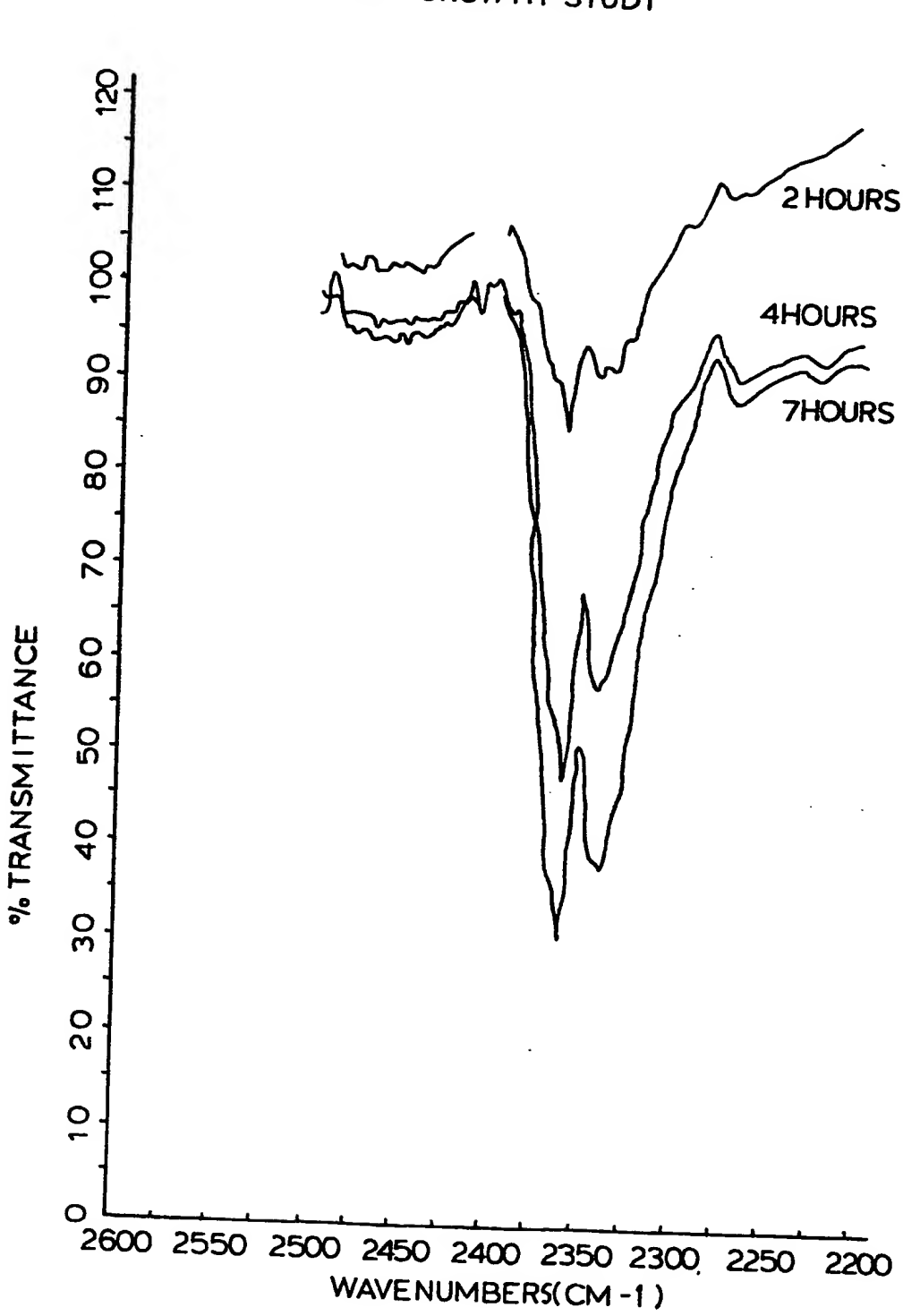

E.COLI GROWTH STUDY
2 HOURS
4HOURS
7HOURS
% TRANSMITTANCE
0
10
20
30
40
50
60
70
80
90
100
110
120
2600 2550 2500 2450 2400 2350 2300 2250 2200
WAVENUMBERS(CM -1)

FIG.8

IR SPECTRUM OF HEAD SPACE GAS FROM:
A. STERILE MEDIUM CONTROL
B. STERILE MEDIUM WITH CLOSTRIDIUM PERFRINGENS

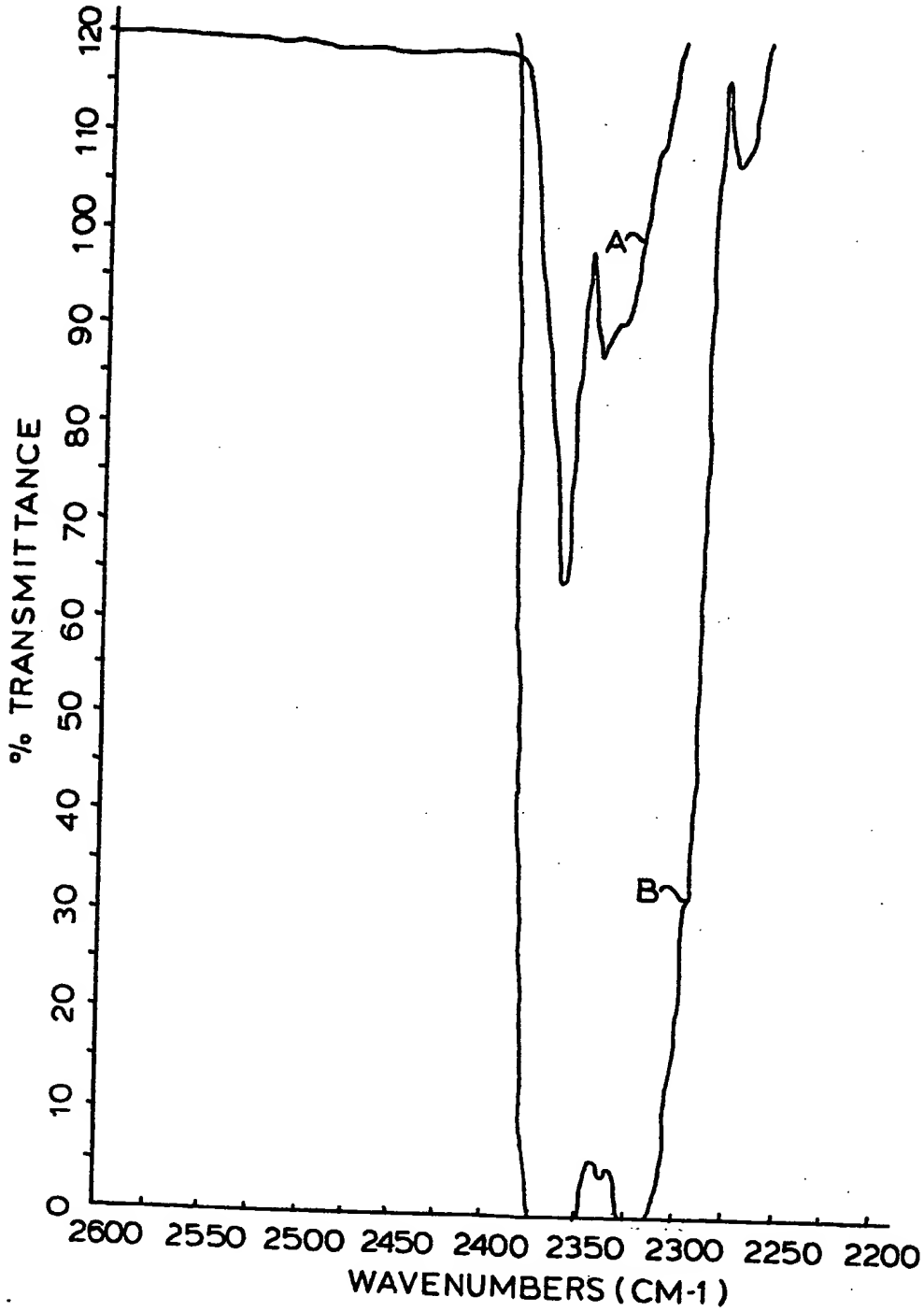
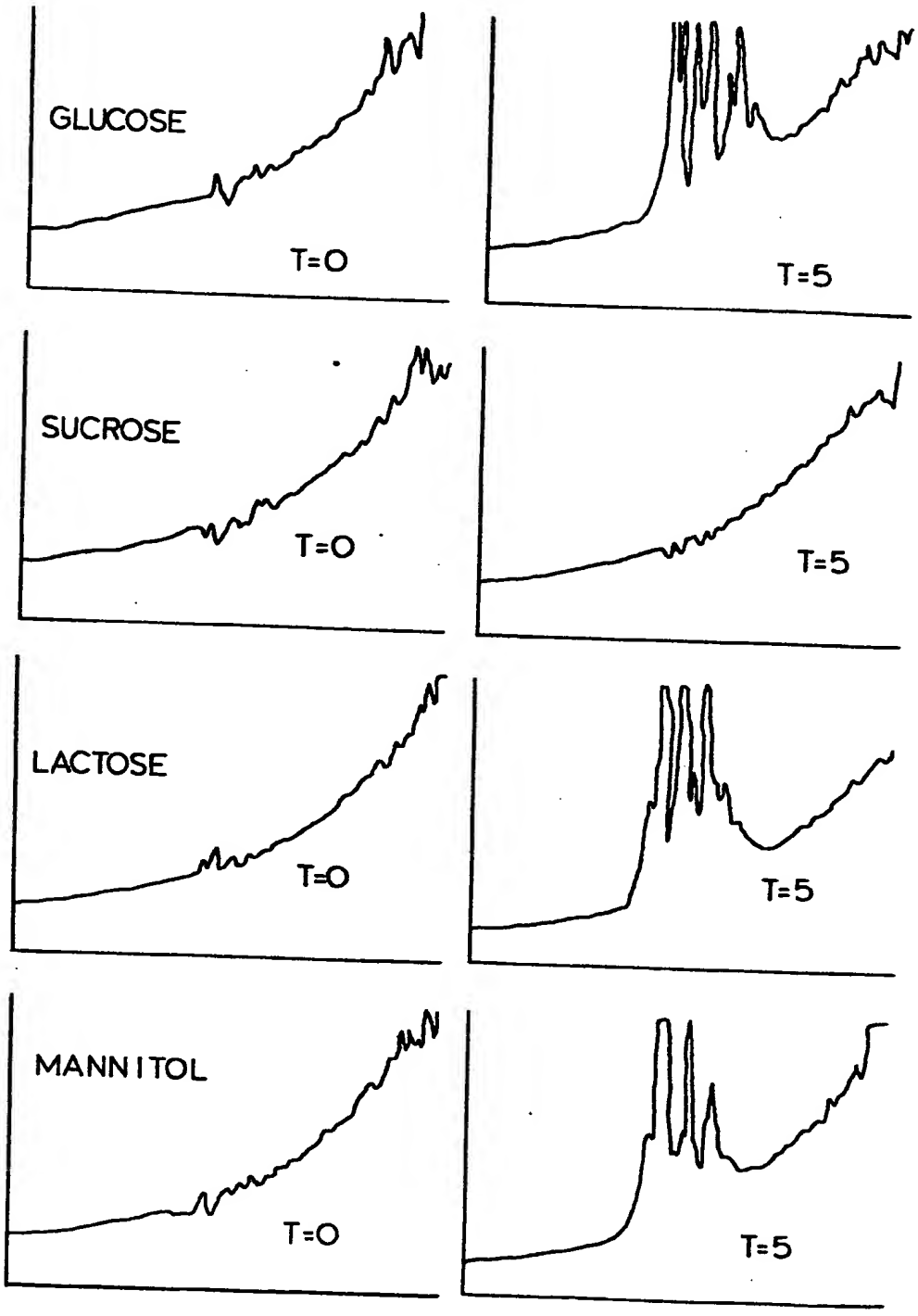
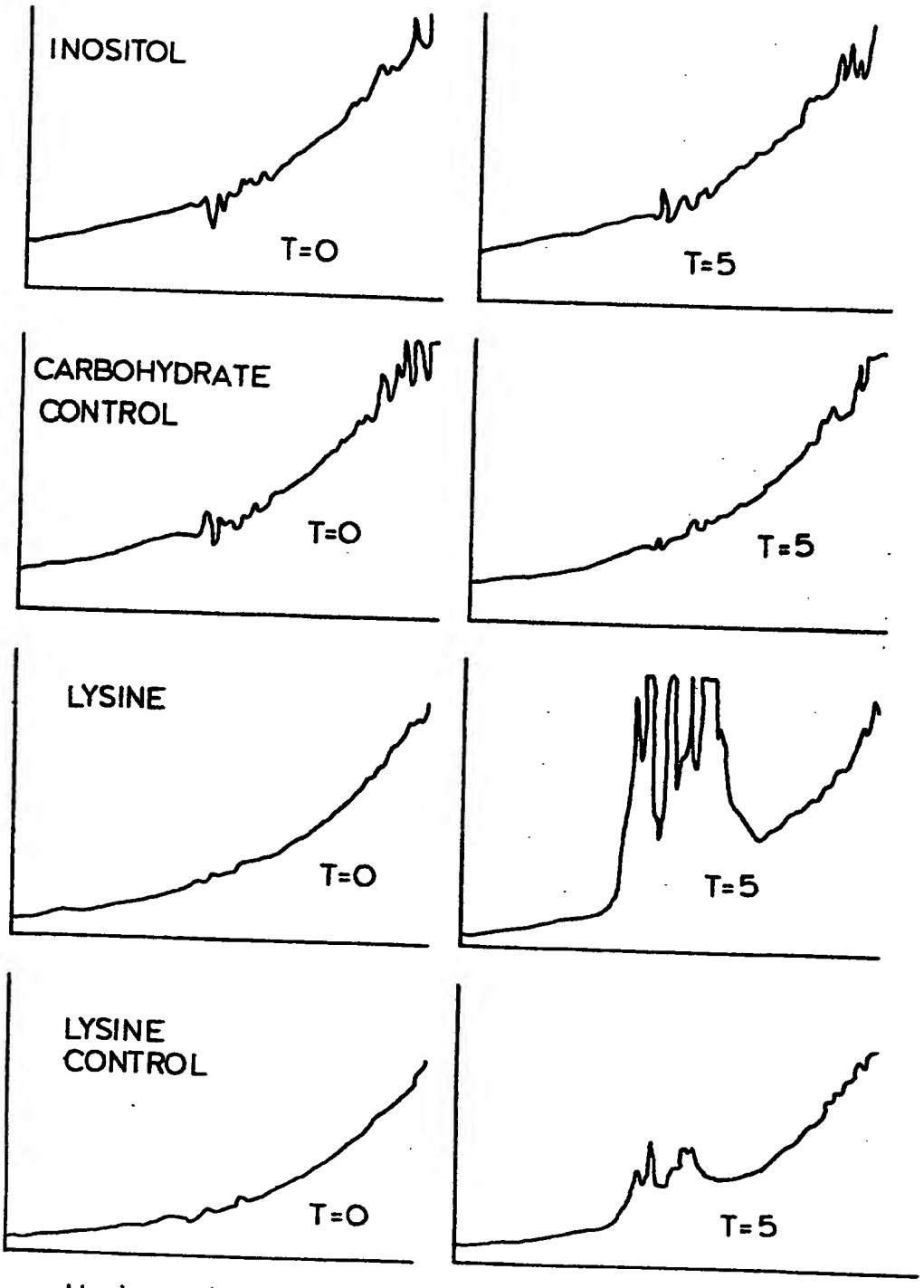


FIG.9



Horizontal axis for all spectra is in wave numbers from 2200 to 2500 cm^{-1}. Vertical axis for all spectra is in absorbance unitsfrom 0.8 to 4.0 .

FIG.10



Horizontal axis for all spectra is in wave numbers from 2200 to 2500 cm^{-1}. Vertical axis for all spectra is in absorbance units from 0.8 to 4.0.

FIG.11

European Patent Office

EUROPEAN SEARCH REPORT

0151855
Application number

EP 84 30 6067

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int Cl 4)
Y,D	JOURNAL OF FOOD SCIENCE, vol. 47, no. 4, July/August 1982, pages 1222-1225, US; C.H. THRELKELD: "Detection of microbial contamination utilizing an infrared CO2 analyzer" * Whole document *	1-20	C 12 M 1/34
Y,D	FR-A-2 256 246 (BIOSPHERICS INC.) * Claims 1-18; page 6, line 4 - page 7, line 20 *	1-20	
P,Y	EP-A-0 104 463 (BECTON, DICKINSON & CIE) * Whole document *	1-20	

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

C 12 M

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	15-05-1985	COUCKE A.O.M.

CATEGORY OF CITED DOCUMENTS

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